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Wilder C.
10/080959

FILE 'REGISTRY' ENTERED AT 13:54:54 ON 10 JUN 2003
L1 76 SEA ABB=ON PLU=ON GTTGCTTCGGCGGGAAC|TTTGCGTTTGCCACTCAGA G|CTGCGCCCGGATCCAGGC/SQSN
L2 11 SEA ABB=ON PLU=ON L1 AND SQL=<25

FILE 'HCAPLUS' ENTERED AT 13:56:34 ON 10 JUN 2003
L3 4 SEA ABB=ON PLU=ON L2

L3 ANSWER 1 OF 4 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:778218 HCAPLUS
DOCUMENT NUMBER: 137:274047
TITLE: Methods for detection of 18S rRNA of Stachybotrys chartarum in pure culture using quantitative polymerase chain reaction
INVENTOR(S): Cruz-Perez, Patricia; Buttner, Mark P.
PATENT ASSIGNEE(S): University of Nevada - Las Vegas, USA
SOURCE: PCT Int. Appl., 40 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002079513	A2	20021010	WO 2002-US6335	20020228
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2003054369	A1	20030320	US 2002-80959	20020222

PRIORITY APPLN. INFO.: US 2001-280712P P 20010329

WO 02079513 Applicant's Work

AB A method for detecting the fungus Stachybotrys chartarum includes isolating DNA from a sample suspected of contg. the fungus Stachybotrys chartarum. The method further includes subjecting the DNA to polymerase chain reaction uing primers for detection of Stachybotrys chartarum 18S rRNA internal transcribed spacer regions. The present invention thus provides protocols for the rapid detection and quantitation of the toxigenic fungus Stachybotrys chartarum by means of polymerase chain reaction (PCR).

IT **466701-34-8 466701-35-9**
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(primer sequence; methods for detection of 18S rRNA of Stachybotrys chartarum in pure culture using quant. polymerase chain reaction)

IT **466701-37-1**
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)

(probe sequence; methods for detection of 18S rRNA of Stachybotrys chartarum in pure culture using quant. polymerase chain reaction)

L3 ANSWER 2 OF 4 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2002:367214 HCAPLUS
DOCUMENT NUMBER: 136:381341
TITLE: Primers and probes for identifying and quantifying specific fungi and bacteria
INVENTOR(S): Haugland, Richard; Vesper, Stephen
PATENT ASSIGNEE(S): U.S. Environmental Protection Agency, USA
SOURCE: U.S., 55 pp., Cont.-in-part of U.S. Ser. No. 290,990, abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6387652	B1	20020514	US 2000-593012	20000613
WO 2001096612	A2	20011220	WO 2001-US18892	20010613

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

WO 2001096612

PRIORITY APPLN. INFO.:
US 1998-81773P P 19980415
US 1999-290990 B2 19990414
US 2000-593012 A2 20000613

AB Fungi and bacteria can be detected and rapidly quantified by using the nucleotide sequences taught here that are specific to the particular species or group of species of fungi or bacteria. Use of the sequences can be made with fluorescent labeled probes, such as in the TaqMan system which produces real time detection of polymerase chain reaction (PCR) products. Other methods of detection and quantification based on these sequences include hybridization, conventional PCR or other mol. techniques. Primers and probes for the detection of the internal transcribed spacers of ribosomal DNAs are described.

IT **424854-63-7**
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(nucleotide sequence, probe for detection of Stachybotris; primers and probes for identifying and quantifying specific fungi and bacteria)

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 3 OF 4 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:924045 HCAPLUS
DOCUMENT NUMBER: 136:49313
TITLE: Method of identifying and quantifying specific fungi and bacteria
INVENTOR(S): Haugland, Richard; Vesper, Stephen Joseph
PATENT ASSIGNEE(S): United States Environmental Protection Agency, USA
SOURCE: PCT Int. Appl., 110 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001096612	A2	20011220	WO 2001-US18892	20010613
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 6387652	B1	20020514	US 2000-593012	20000613

PRIORITY APPLN. INFO.: US 2000-593012 A2 20000613
US 1998-81773P P 19980415
US 1999-290990 B2 19990414

AB Fungi and bacteria can be detected and quantified by using a nucleotide sequence taught here that are specific to the particular species or group of species of fungi or bacteria. Use of the sequences can be made with fluorescent labeled probes, such as in the TaqMantrade; system which produces real time detection of polymerase chain reaction (PCR) products. Other methods of detection and quanitification based on these sequences include hybridization, convention PCR or other mol. techniques.

IT 382674-25-1
RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); PRP (Properties); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(method of identifying and quantifying specific fungi and bacteria)

L3 ANSWER 4 OF 4 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2001:345190 HCAPLUS
DOCUMENT NUMBER: 136:145631
TITLE: Specific detection of Stachybotrys chartarum in pure culture using quantitative polymerase chain reaction
AUTHOR(S): Cruz-Perez, P.; Buttner, M. P.; Stetzenbach, L. D.
CORPORATE SOURCE: Harry Reid Center for Environmental Studies, University of Nevada, Las Vegas, NV, 89154-4009,

USA
SOURCE: Molecular and Cellular Probes (2001), 15(3), 129-138
CODEN: MCPRE6; ISSN: 0890-8508
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Research was conducted with lab. cultures to establish a protocol for the rapid detection and quantitation of the toxigenic fungus Stachybotrys chartarum by means of polymerase chain reaction (PCR). Sequences for the 18 S rRNA gene of S. chartarum were obtained from GenBank and compared against all other available sequences online with the Basic Local Alignment Search Tool (BLAST). Two sets of TaqManTMprimers and one fluorescently labeled probe were designed and tested for selectivity, specificity and sensitivity of detection. A fluorogenic nuclease assay in conjunction with a sequence detector were used for the amplification and quantitation of S. chartarum. The primers designed amplified all S. chartarum isolates tested and did not amplify DNA extd. from other Stachybotrys species or 15 other fungal genera. The primer set selected had a sensitivity of <23 template copies. Many S. chartarum samples were initially neg. after PCR amplification. Incorporation of an internal pos. control in the PCR reaction demonstrated the presence of inhibitors in these samples. PCR inhibitors were removed by diln. or further purifn. of the DNA samples. The results of this research report on a quant. PCR (QPCR) method for detection and quantitation of S. chartarum and demonstrate the presence of PCR inhibitors in some S. chartarum isolates. (c) 2001 Academic Press.

IT **395174-30-8D**, 5' 6-FAM and 3' TAMRA labeled
RL: ANT (Analyte); PRP (Properties); ANST (Analytical study)
(nucleotide sequence for probe; specific detection of Stachybotrys chartarum in pure culture using quant. polymerase chain reaction)

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

E1 THROUGH E6 ASSIGNED

FILE 'REGISTRY' ENTERED AT 13:57:50 ON 10 JUN 2003
L4 6 SEA FILE=REGISTRY ABB=ON PLU=ON (382674-25-1/BI OR 395174-30-8/BI OR 424854-63-7/BI OR 466701-34-8/BI OR 466701-35-9/BI OR 466701-37-1/BI)

L5 6 L2 AND L4

L5 ANSWER 1 OF 6 REGISTRY COPYRIGHT 2003 ACS
RN **466701-37-1** REGISTRY
CN DNA, d(C-T-G-C-G-C-C-C-G-G-A-T-C-C-A-G-G-C) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN 5: PN: WO02079513 SEQID: 5 claimed DNA
CI MAN
SQL **18**

SEQ 1 ctgcgcccgg atccaggc
========== ========
HITS AT: 1-18

RELATED SEQUENCES AVAILABLE WITH SEQLINK

REFERENCE 1: 137:274047

L5 ANSWER 2 OF 6 REGISTRY COPYRIGHT 2003 ACS
RN **466701-35-9** REGISTRY
CN DNA, d(T-T-T-G-C-G-T-T-T-G-C-C-A-C-T-C-A-G-A-G) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN 2: PN: WO02079513 SEQID: 2 claimed sequence
CI MAN
SQL **20**

SEQ 1 tttgcgtttg ccactcagag
========== ==========
HITS AT: 1-20

RELATED SEQUENCES AVAILABLE WITH SEQLINK

REFERENCE 1: 137:274047

L5 ANSWER 3 OF 6 REGISTRY COPYRIGHT 2003 ACS
RN **466701-34-8** REGISTRY
CN DNA, d(G-T-T-G-C-T-T-C-G-G-C-G-G-G-A-A-C) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN 1: PN: WO02079513 SEQID: 1 claimed sequence
CI MAN
SQL **17**

SEQ 1 gttgcttcgg cgggaac
========== =======
HITS AT: 1-17

RELATED SEQUENCES AVAILABLE WITH SEQLINK

REFERENCE 1: 137:274047

L5 ANSWER 4 OF 6 REGISTRY COPYRIGHT 2003 ACS
RN **424854-63-7** REGISTRY
CN DNA, d(C-T-G-C-G-C-C-C-G-G-A-T-C-C-A-G-G-C) (9CI) (CA INDEX NAME)
OTHER NAMES:
CN 189: PN: US6387652 SEQID: 188 claimed DNA
CI MAN
SQL **18**

SEQ 1 ctgcgcccgg atccaggc
========== ========
HITS AT: 1-18

RELATED SEQUENCES AVAILABLE WITH SEQLINK

REFERENCE 1: 136:381341

L5 ANSWER 5 OF 6 REGISTRY COPYRIGHT 2003 ACS
RN **395174-30-8** REGISTRY
CN DNA, d(C-T-G-C-G-C-C-C-G-G-A-T-C-C-A-G-G-C) (9CI) (CA INDEX NAME)
CI MAN

SQL **18**

SEQ 1 ctgcgcccgg atccaggc
========== ========
HITS AT: 1-18

RELATED SEQUENCES AVAILABLE WITH SEQLINK

REFERENCE 1: 136:145631

L5 ANSWER 6 OF 6 REGISTRY COPYRIGHT 2003 ACS
RN **382674-25-1** REGISTRY
CN 188: PN: WO0196612 SEQID: 188 claimed DNA (9CI) (CA INDEX NAME)
CI MAN
SQL **18**

SEQ 1 ctgcgcccgg atccaggc
========== ========
HITS AT: 1-18

RELATED SEQUENCES AVAILABLE WITH SEQLINK

REFERENCE 1: 136:49313

FILE 'HOME' ENTERED AT 13:58:10 ON 10 JUN 2003

Detecting and quantifying fungi and bacteria, involves obtaining a sequence of the fungus, extracting the DNA from the sample, and subjecting the DNA to **polymerase chain reaction** and fluorescent probe analysis;

Stachybotrys chartarum conidia detection using DNA sequence-specific DNA primer and DNA probe in real-time **polymerase chain reaction** analysis

AUTHOR: HAUGLAND R; VESPER S J
PATENT ASSIGNEE: US ENVIRONMENTAL PROTECTION AGENCY
PATENT INFO: WO 2001096612 20 Dec 2001
APPLICATION INFO: WO 2000-US18892 13 Jun 2000
PRIORITY INFO: US 2000-593012 13 Jun 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-098078 [13]
AN 2002-07556 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Detecting and quantifying (M1) fungi and bacteria, involves obtaining a sequence of the microorganism to be detected and quantified, extracting the DNA from the sample, and subjecting the DNA to **polymerase chain reaction** (**PCR**) and fluorescent probe analysis.

BIOTECHNOLOGY - Preferred Microorganism: The fungi and bacteria are selected from any one of the microorganisms given in the specification. Preferred Method: The label used in (M1) is a fluorescent label. The microorganism is detected and quantitated using **PCR**, hybridization or other molecular techniques. The primers and probes given in the specification are used for determining the cell quantities of fungi and bacteria. There were over 200 probes and primer sequences claimed in the specification which are specific for detecting a particular microorganism.

USE - (M1) is useful for identifying and quantifying specific fungi and bacteria using specific DNA sequences. The specific DNA sequences are useful for the real time detection of **PCR** products with a fluorogenic probe system or other molecular probes like hybridization.

ADVANTAGE - The method is simple and reliable.

EXAMPLE - Conidial stocks of the target fungus, **Stachybotrys chartarum**, and the **reference** target, Geotrichum candidum, were prepared to act as calibrator and internal **standard**, respectively. Genomic DNAs were extracted from 20 microlitres conidial suspensions using a glass bead milling and glass milk adsorption method. Briefly, the method involved mixing test and **reference** conidia suspensions with 0.3 g of acid-washed glass beads and 10 microlitres, 100 microlitres and 300 microlitres, respectively, of glass milk suspensions, lysis buffer and binding buffer in sterile 2 ml conical bottom, screw cap tubes. The tubes were shaken in a mini beadbeater for one minute at maximum rate and DNAs were recovered in final volumes of 200 microlitres distilled water. TaqMan probes containing a TAMRA group conjugated to their 3'-terminal nucleotide and a FAM group linked to their 5'-terminal nucleotides as the quencher and reporter fluorochromes, respectively, and TaqMan primers were obtained. For G. candidum, the primers used were NS92F (5'-CACCGCCCGTCGCTAC) and GcandR1 (5'-AGAAAAGTTGCCCTCTCCAGTT), and the probe was GeoP2

(5'-TCAATCCGGAAGCCTCACTAAGCCATT). For S. chartarum, the primers used were StacF4 (5'-TCCCAAACCCTTATGTGAACC) and StacR5 (5'-GTTTGCCACTCAGAGAATACTGAAA), and the probe was StacP2 (5'-CTGCGCCCGGATCCAGGC). **Polymerase chain reaction** (**PCR**) reactions were prepared in 0.5 ml thin-walled, optical grade **PCR** tubes. Assays for S. chartarum and G. candidum sequences in the same DNA samples were performed in separate reaction tubes. Quantification of S. chartarum conidia using the comparative CT method was performed by first subtracting mean **reference** sequence CT values from mean target sequence CT values for both test samples and a pre-specified calibrator sample to obtain (DELTA)CT values. Calibrator sample (DELTA)CT values were then subtracted from (DELTA)CT values of the test samples to obtained (DELTA)(DELTA)CT valued. Calibrator samples were DNA extracts from mixtures of approximately 2 x 10 (to the powr of 4) S. chartarum (strain UMAH 6417) and 2 x 10 (to the powr of 5) G. candidum conidia. Test samples were mixed with the same quantity of G. candidum conidia prior to DNA extraction. Ratios of target sequences determined in the test and calibrator samples were then multiplied by the known quantities of S. chartarum conidia in the calibrator samples to obtain estimates of the absolute quantities of these conidia in the test samples.(110 pages)

(FILE 'HOME' ENTERED AT 11:28:33 ON 12 JUN 2003)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
ENTERED AT 11:28:48 ON 12 JUN 2003

```
L1          600 S STACHYBOTRYS CHARTARUM
L2         2041 S STACHYBOTRYS
L3          267 S (L1 OR L2) AND (STANDARD OR CONTROL OR REFERENCE)
L4           36 S L3 AND (PCR OR POLYMERASE CHAINR REACTION OR AMPLIF?)
L5           36 S L3 AND (PCR OR POLYMERASE CHAIN REACTION OR AMPLIF?)
L6           17 DUP REM L5 (19 DUPLICATES REMOVED)

=>
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